Cow's Milk Consumption, Disease-Associated Autoantibodies and Type 1 Diabetes Mellitus: a Follow-up Study in Siblings of Diabetic Children

S.M. Virtanen*[1], E. Hyppönen[1], E. Läärä[2], P. Vähäsalo[3], P. Kulmala[3], K. Savola[3], L. Räsänen[4], A. Aro[5], M. Knip[3,6], H.K. Åkerblom[7], and the Childhood Diabetes in Finland Study Group†

[1]School of Public Health, University of Tampere, Tampere, Finland
[2]Department of Mathematical Sciences, University of Oulu, Oulu, Finland
[3]Department of Pediatrics, University of Oulu, Oulu, Finland
[4]Division of Nutrition, University of Helsinki, Helsinki, Finland
[5]Department of Nutrition, National Public Health Institute, Helsinki, Finland
[6]Medical School, University of Tampere, Tampere, Finland
[7]The Children's Hospital, University of Helsinki, Helsinki, Finland

Evidence from case–control studies for the diabetogenicity of introduction of cow's milk-based formulas at early age in infancy is inconclusive. We followed siblings of children with Type 1 diabetes mellitus (Type 1 DM) to investigate a possible relationship between cow's milk consumption during infancy or later in childhood and the emergence of diabetes-associated autoantibodies and progression to clinical Type 1 DM. A cohort of 725 initially unaffected 0 to 25-year-old siblings of 801 index children with Type 1 DM diagnosed in 1986–1989 participated in the study (82 % of those invited). The siblings were observed for Type 1 DM associated autoantibodies at intervals of 3–12 months for 4 years, starting from the diagnosis of Type 1 DM in the index child. The follow-up for Type 1 DM started at the same time and ended on 31 October 1995. The combined prevalence of Type 1 DM associated autoantibodies (islet cell antibodies (ICA), insulin autoantibodies (IAA), GAD autoantibodies (GADA), and/or antibodies to the insulinoma associated cDNA2 protein (IA-2A)) was 13.6 % (95/697) at the beginning of the study. Of the initially seronegative siblings, 7.5 % (45/602) converted to antibody positivity during 4 years, and of all siblings 4.6 % (33/725) developed Type 1 DM during the total follow-up time. The age at introduction of supplementary milk feeding was not significantly related to seroconversion to positivity for Type 1 DM associated autoantibodies or to the development of Type 1 DM in the siblings. When adjusted for age, sex, infant feeding patterns, and maternal age and education, high milk consumption in childhood ($\geq$3 glasses daily) was associated with more frequent emergence of Type 1 DM-associated autoantibodies than low consumption ($<$3 glasses daily) (adjusted odds ratio 3.97, 95 % confidence interval 1.3–11.7, $p = 0.01$). There was a non-significant association between high milk consumption and progression to clinical Type 1 DM (adjusted hazard ratio 2.75, 95 % confidence interval 0.9–8.4, $p = 0.07$). To conclude, this study suggests that high consumption of cow's milk during childhood may be associated both with seroconversion to positivity for diabetes-associated autoantibodies and progression to clinical Type 1 DM among siblings of children with diabetes.

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Abbreviations: CI confidence interval, GADA GAD autoantibody, HR hazard ratio, IAA insulin autoantibody, IA-2A antibody to the insulinoma associated cDNA2 protein, ICA islet cell antibody, OR odds ratio

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* Correspondence to: Dr. Suvi Virtanen, School of Public Health, University of Tampere, PO Box 607, FIN-33101 Tampere, Finland. E-mail: mesuri.uta.fi

†Members of the Childhood Diabetes in Finland Study Group: *Principal investigators* H.K. Åkerblom and J. Tuomilehto; *Co-ordinators* R. Lounamaa, L. Toivanen; *Data management* J. Pitkäniemi, E. Virtala; *Local investigators.* A. Fagerlund, M. Flittner, B. Gustafsson, C. Häggqvist, A. Hakulinen, L. Herva, P. Hiltunen, T. Huhtamäki, N.-P. Huttunen, T. Huupponen, M. Hyttinen, T. Joki, R. Jokisalo, M.-L. Käär, S. Kallio, E.A. Kaprio, U. Kaski, M. Knip, L. Laine, J. Lappalainen, J. Mäenpää, A.-L. Mäkelä, K. Niemi, A. Niiranen, P. Ojajärvi, T. Otonkoski, K. Pihlajamäki, S. Pöntynen, J. Rajantie, J. Sankala, J. Schumacher, M. Sillanpää, M.-R. Ståhlberg, C.-H. Stråhlmann, T. Uotila, M. Väre, P. Varimo, and C. Wetterstrand; *Special investigators* A. Aro, M. Hiltunen, H. Hurme, H. Hyöty, J. Ilonen, J. Karjalainen, M. Knip, P. Leinikki, A. Miettinen, T. Petäys, L. Räsänen, H. Reijonen, A. Reunanen, T. Saukkonen, E. Savilahti, E. Tuomilehto-Wolf, P. Vähäsalo, S.M. Virtanen

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Introduction

The incidence of Type 1 diabetes mellitus (Type 1 DM) in children in Finland is the highest in the world. It has more than tripled since the early 1950s,[1,2] which suggests that environmental factors play a major role in this increase. There have been suggestions that cow's milk may be diabetogenic in humans.[3] Case–control studies indicated that the introduction of cow's milk based formulas at an early age increases the risk of Type 1 DM,[4,5] independently of the duration of breast-feeding.[6] Altered immune responses to cow's milk proteins have been observed in children with newly diagnosed Type 1 DM.[7] Humoral immune responses to cow's milk proteins were related not only to age at introduction of supplementary milk feeding in infancy,[8,9] but also to milk consumption later in childhood.[8] Ecological studies have shown high positive associations between *per capita* cow's milk consumption and the risk of Type 1 DM in children in several industrialized countries.[10,11] Cow's milk protein has been observed to be diabetogenic in BB rats and NOD mice.[12,13] In Finland, customary milk consumption among children and adolescents has traditionally been,[14] and continues to be, high, between half and one litre per day. To evaluate the role of cow's milk consumption both during infancy and childhood on the development of Type 1 DM in a prospective design, we followed siblings of children with Type 1 DM for diabetes-associated autoantibodies and for the manifestation of clinical diabetes.

Subjects and Methods

Subjects

In the nationwide 'Childhood Diabetes in Finland' study on genetic and environmental determinants of childhood Type 1 DM,[15] detailed data were collected on infant feeding patterns and on diet in later childhood, and sequential blood samples were taken for the analyses of Type 1 DM associated autoantibodies.[6,16] During the recruitment period, from September 1986 to April 1989, 801 families with a newly diagnosed child with Type 1 DM were invited to participate in the study, including all unaffected siblings between 3 and 19 years of age ($n = 819$). Of the siblings, 82 % participated in the dietary survey, and the first blood sample was obtained from 82 % of the siblings (at the time of diagnosis of Type 1 DM in the index child). Dietary data were also available for 37 siblings younger than 3 years of age and for 8 older than 19 years. From these, 28 and 4, respectively, gave serum samples. Altogether dietary data were available from 725 siblings, who form the present study population. Autoantibody analysis at the beginning of the study were available from 697 siblings. The median age of the siblings at recruitment was 9.4 (range 0.4–24.9) years. Only 1 sibling participated from 324 families, 2 from 138 families, 3 from 25 families, 4 from 6 families, 6 from 3 families, and from 1 family there were 8 participating siblings.

Diet and Sociodemographic Characteristics

Dietary and sociodemographic data were collected by structured questionnaires at the time of diagnosis of Type 1 DM in the index child.[4] Of the siblings, 12 % filled in the questionnaire themselves and 7 % with the help of an adult, but otherwise the mother (73 %), the father (4 %) or somebody else (4 %) filled in the questionnaire. In the present study, the following infant feeding variables were used: the duration of overall (total) breast-feeding (including any time when the child received other foods in addition) and the age at introduction of cow's milk products to the diet (infant formulas, cow's milk, other cow's milk products). The combined usual amount of milk or sour milk consumed daily (in glasses, 1 glass = 180 g), and consumption frequencies of milk, sour milk, yogurt, ice cream, and cheese during a half-year period before entering the study were asked. The sociodemographic variables used in the present study were the child's age and sex, and the length of education of the mother and maternal age at the beginning of the study (at the time of diagnosis of Type 1 DM in the index child).

Outcome and Follow-up Measures

As outcome measures we used:

1. seroconversion to positivity for at least one out of four disease associated autoantibodies (islet cell antibodies (ICA), insulin autoantibodies (IAA), GAD autoantibodies (GADA), and antibodies to the insulinoma associated cDNA2 protein (IA-2A)) on at least one occasion during the 4-year follow-up period from the diagnosis of Type 1 DM in the index child;
2. presentation with clinical Type 1 DM over the time period from the diagnosis of Type 1 DM in the index child until 31 October 1995. Initial blood samples for the analyses of ICA, IAA, GADA, and IA-2A in the siblings were taken within the first 1–3 weeks (median 2 weeks) after the diagnosis of Type 1 DM in the index child, except in a small number of siblings in whom the first sample was obtained later during the first 6 months ($n = 90$, 13 %). Blood samples from the siblings were collected at intervals of 3–6 months over the initial 2 years, and subsequently annually up to 4 years from the diagnosis of Type 1 DM in the index child: median 9 (range 2–18) blood samples from different time points were available.

Information on the newly diagnosed cases of Type 1 DM were obtained from the Central Drug Registry of the Finnish Social Insurance Institution as described.[17] The ethical committees of all the participating hospitals

had approved the study protocol. Informed consent was obtained from the participating families.

Analyses

ICAs were determined by a standard immunofluorescence method using sections of frozen human group O pancreas.[18] End point dilution titres were examined for positive samples and the results were expressed in JDF units relative to an international reference standard.[19] The detection limit was 2.5 JDF units. The inter-assay variation has been found to be maximally one dilution step without any overlapping between positive and negative samples. Our laboratory has participated in international workshops on the standardization of the ICA assay, in which its sensitivity was 100 %, specificity 98 %, validity 98 %, and consistency 98 % in the most recent round.

GADA antibodies were measured with a radioligand assay as described by Petersen *et al.*[20] Recombinant human islet GAD65 cDNA was transcribed and translated *in vitro* according to the manufacturer's instructions (Promega, Madison, WI, USA). Serum samples (final dilution 1:25) were incubated overnight with approximately 30 000 cpm of ^{35S}-methionine-labelled *in vitro* translated human GAD65 in a total volume of 50 μl Tris-buffered saline with Tween (TBST). Protein-A-Sepharose (7.5 mg, Pharmacia, Uppsala, Sweden) in a total volume of 100 μl TBST was used to isolate the immunocomplexes. The quantity of precipitated immunocomplexes was counted with a scintillation counter. All the samples were analysed in quadruplicate with and without competition from an excess of unlabelled purified human recombinant GAD65 (1 μg well^{-1}) produced in baby hamster kidney cells. The results were expressed in relative units, representing the specific binding as a percentage of that obtained with a positive standard serum. The cut-off limit for antibody positivity was set at the 99th percentile in 372 non-diabetic children and adolescents, i.e. 6.6 relative units. The intra-assay coefficient of variation was less than 5 %, and the inter-assay coefficient less than 10 % at a GADA level of 95 RU and 15 % at a GADA level of 7.3 RU. The disease sensitivity of the present assay was 80 % and the specificity 94 %, based on the 101 samples included in the Second International GAD Antibody Workshop.[21]

IAA levels were analysed with a radiobinding assay modified from that described by Palmer *et al.*[22] Endogenous insulin was removed with acid charcoal prior to the assay, and free and bound insulin were separated after incubation with mono -^{125}I(Tyr A 14)- human insulin (Novo Research Institute, Bagsvaerd, Denmark) for 20 h in the absence or presence of an excess of unlabelled insulin. The IAA levels were expressed in nU ml^{-1}, where 1 nU ml^{-1} corresponds to a specific binding of 0.01 % of the total counts. The inter-assay coefficient of variation was less than 8 % at an IAA level of 1280 nU ml^{-1}. At an IAA level of 60 nU l^{-1} the inter-assay variation was 14 %. A subject was considered to be positive for IAA when the specific binding exceeded 54 nU ml^{-1} (99th percentile in 105 non-diabetic subjects).

IA-2A were analysed with a radiobinding assay modified from that described by Bonifacio *et al.*[23] Briefly, recombinant cDNA plasmid encoding the intracellular part of the full length IA-2A protein was multiplied in *E. coli* JM109 and then purified with standard techniques. The IA-2A antigen was produced with *in vitro* transcription and translation of purified cDNA by the TNT® Coupled Reticulocyte Lysate Systems (Promega, Madison, WI, USA) in the presence of ^{35}S-methionine (Amersham, Amersham, Bucks, UK) according to the manufacturer's instructions. Unincorporated ^{35}S-methionine was removed by gel chromatography on a NAP5 column (Pharmacia, Uppsala, Sweden). Two μl of serum samples were incubated at +4 °C overnight with 10 000 cpm of labelled IA-2A diluted in 50 μl of 50 mM Tris, 150 mM NaCl, (pH 7.4) containing 0.1 % Tween®20 (TBST). On the next day 5 μl Protein-A-Sepharose® CL-4B (Pharmacia) in a total volume of 50 μl TBST was added to isolate immune complexes. After incubation for at least 1 h on a shaker at +4 °C samples were washed 10 times with approximately 150 μl TBST using a vacuum device (Millipore, Bedford, MA, USA). The activity of samples was measured in a liquid scintillation counter (1450 Microbeta® Trilux, Wallac, Turku, Finland). All samples were tested in duplicate. The results were expressed as relative units based on a standard curve run on each plate (MultiCalc™, Wallac). The limit for positivity (0.43 relative units) was set at the 99th percentile of 374 Finnish children and adolescents without diabetes. The inter-assay variation of the IA-2A assay was 8 % at an IA-2A level of 82.6 RU and 12 % at a level of 0.63 RU. The disease sensitivity of the assay was 62 % and specificity 97 % based on 140 samples included in the Multiple Autoantibody Workshop.

Statistical Analyses

The incidence of seroconversion to positive antibody status and the incidence of Type 1 DM, in relation to the nutritional factors under study, were analysed by appropriate modifications of generalized linear models.[24] Logistic regression models were fitted to the cumulative incidence of seroconversion between the baseline and the 4-year follow-up antibody assessments in the initially seronegative study subjects.

Estimates of the relative odds of seroconversion associated with the key exposure variables were calculated, each adjusted for some sociodemographic determinants (age and sex of the child, and age and years of education of the mother). Adjusted estimates for the risk factors were also calculated by including other nutritional factors along with the sociodemographic factors simultaneously in the model. Log-linear Poisson models were fitted in the whole population of initially disease-free siblings to their incidence rate of Type 1 DM during the variable

follow-up time, providing analogously adjusted estimates of relative hazard of diabetes for the risk factors of interest. As it is reasonable to assume that the responses of children from the same family are not statistically independent but positively associated, standard maximum-likelihood estimation procedures are not entirely appropriate, because they at least tend to provide standard errors for the model coefficients that are too small. Therefore, we estimated the regression parameters, both in the binary logistic and in the Poisson log-linear model, by the generalized estimating equation approach.[25] In this analysis we assumed a uniform (or exchangeable) working correlation structure within families but independence between families, which is a common practice in similar sibship studies. As there were very few families with more than four children in the study population, we chose by random sampling only four children from these families to be included in these analyses in order to retain computational stability in the estimation of the correlation matrix. The confidence intervals for the relative risk parameters were obtained from the robust variance–covariance estimates of the model coefficients. All computations were performed using the Genmod procedure of the SAS/STAT Software, version 6.12.[26]

Results

Dietary Characteristics of the Study Population

Table 1 shows the distribution of infant feeding variables and childhood milk and sour milk consumption of the siblings. Women who had remained longer in education had breast-fed their children longer than those with a shorter education (Table 2). The duration of overall breast-feeding was longer and the age at introduction of supplementary milk feeding higher, the younger the child was at the time of the study. Older mothers had breast-fed their children longer and had given supplementary milk feeding later compared with the younger ones. Boys consumed more milk and sour milk than girls. The consumption frequencies of other milk products were similar for boys and girls. Of the siblings, 86 % consumed milk, 18 % sour milk products, 16 % cheese, and 1 % ice cream daily.

Disease Associated Autoantibodies and Type 1 DM

At the start of the study (diagnosis of Type 1 DM in the index child), 56 of the 697 tested siblings were positive for ICA, 25 for IAA, 55 for GADA, and 42 for IA-2A. Overall, 95 (13.6 %) were positive for at least 1 of the 4 autoantibodies: 49 were positive for 1, 14 for 2, 27 for 3, and 5 for 4 autoantibodies.

During the 4-year follow-up, 26 of the 602 initially autoantibody negative had seroconverted to positivity for ICA, 9 for IAA, 12 for GADA, and 4 for IA-2A. Altogether 45 (7.5 %) had seroconverted to positivity for at least 1 of the 4 autoantibodies: 40 turned positive for 1 autoantibody, 4 for 2, and 1 for 3.

By 31 October 1995, 33 of 725 initially non-diabetic siblings had progresssed to Type 1 DM. The average follow-up of the siblings was 7.5 (SD 1.3) years from the diagnosis of Type 1 DM in the index child until 31 October 1995. The median interval between the development of Type 1 DM in the index child and in the sibling was 3.6 (range 0.01–7.7) years.

Dietary Factors Related to Disease Associated Autoantibodies and Type 1 DM

The cumulative incidence of Type 1 DM associated autoantibodies and the incidence of Type 1 DM in different categories of dietary variables are presented in Table 1 and in different categories of potential confounders in Table 3.

The duration of total breast-feeding and age at introduction of supplementary milk feeding were not significantly related to seroconversion to positivity for Type 1 DM associated autoantibodies or to progression to clinical disease (Table 4).

The estimated relative risk between high ($\geq$3 glasses day^{-1}) and low ($<$3 glasses day^{-1}) levels of milk consumption was for the risk of seroconversion to positivity for disease-associated autoantibodies 2.21 (95 % confidence interval, CI, 0.9 to 5.2, $p = 0.07$), and for the risk of clinical Type 1 DM 3.11 (95 % CI 1.1 to 9.3, $p = 0.04$), when adjusted for age and sex of the child and maternal age and education (Table 4). When adjusted in addition to the before mentioned sociodemographic factors for infant feeding patterns, the risk ratios were 3.97 (95 % CI 1.3 to 11.7) for seroconversion ($p = 0.01$) and 2.75 (95 % CI 0.9 to 8.4) for progression to clinical Type 1 DM ($p = 0.07$) (Table 4).

The length of maternal education was positively related to the risk of clinical Type 1 DM, but not to the risk of seroconversion to positivity for Type 1 DM-associated autoantibodies (Table 4). Maternal age was not associated with the risk of seroconversion to positivity or with the risk of Type 1 DM when adjusted for maternal education.

Daily consumption of sour milk products was not associated with the risk of developing disease-associated autoantibodies or Type 1 DM, nor was cheese consumption associated with these outcomes (data not shown).

Discussion

In a prospective follow-up of siblings of children with Type 1 DM, we found some, albeit still inconclusive, evidence that cow's milk consumption during childhood (but not in infancy) was associated with the emergence of disease-associated autoantibodies and further with progression to clinical Type 1 DM. This association was

Table 1. Distribution of dietary variables and the prevalence and cumulative incidence of Type 1 DM associated autoantibodies and incidence rate of Type 1 DM in the siblings of children with diabetes

	Number (%) of children	Type 1 DM associated autoantibodies[a]		Type 1 DM
		Prevalence[b] per 100 at baseline (cases)	Cumulative incidence[c] per 100 (cases)	Incidence rate[d] per 1000 yr (cases)
Duration of total breast-feeding (months)				
<2	91 (13)	15.6 (14)	10.5 (8)	7.1 (5)
≥2	589 (81)	13.3 (75)	7.1 (35)	6.4 (28)
Missing	45 (6)	14.3 (6)	5.6 (2)	0 (0)
Age at introduction of milk supplements (months)				
<2	157 (22)	14.2 (22)	6.0 (8)	7.7 (9)
≥2	494 (68)	13.0 (61)	7.3 (30)	5.7 (21)
Missing	74 (10)	16.9 (12)	11.9 (7)	5.3 (3)
Childhood milk and sour milk consumption (glass day^{-1})				
0	56 (8)	7.1 (4)	6.4 (3)	0 (0)
1–2	189 (26)	12.8 (23)	4.5 (7)	2.8 (4)
3–4	325 (45)	14.3 (45)	9.6 (26)	8.7 (21)
≥5	148 (20)	14.6 (21)	7.3 (9)	5.5 (6)
Missing	7 (1)	28.6 (2)	0 (0)	56.0 (2)
All	725 (100)	13.6 (95)	7.5 (45)	6.1 (33)

[a]Autoantibodies measured from 697 children.
[b]For disease-associated autoantibodies at the time of diagnosis of Type 1 DM in the index child ($n = 697$). [c]For disease-associated autoantibodies follow-up from the diagnosis of Type 1 DM in the index child over a period of 4 years, among those initially seronegative ($n = 602$).
[d]For development of Type 1 DM observation from the diagnosis of Type 1 DM in the index child until 31 October 1995 ($n = 725$).

Table 2. Infant feeding patterns and childhood milk and sour milk consumption by sociodemographic factors in the siblings of children with diabetes

	Duration of total breast-feeding (months) median (Q1, Q3)[a]	Age at introduction of milk supplements (months) median (Q1, Q3)[a]	Childhood milk and sour milk consumption (glass day^{-1}) median (Q1, Q3)[a]
Boys ($n = 338$)	5 (2,10)	3 (1,7)	4 (2,5)
Girls ($n = 387$)	5 (3,9)	3 (2,7)	3 (2,4)
Age groups (yr)			
0–4 ($n = 134$)	9 (6,13)	7 (4,9)	3 (2,4)
5–9 ($n = 259$)	7 (4,11)	5 (2,8)	3 (2,4)
10–14 ($n = 205$)	3 (2,6)	3 (1,4)	3 (2,5)
15–24 ($n = 127$)	3 (2,4)	2 (1,3)	3 (2,4)
Maternal age (yr)			
17–22 ($n = 148$)	3 (2,6)	3 (2,5)	3 (2,4)
23–29 ($n = 373$)	4 (3,9)	3 (1,7)	3 (2,4)
30–44 ($n = 203$)	7 (3,11)	6 (2,8)	3 (2,4)
Length of maternal education (yr)			
6–9 ($n = 198$)	3 (2,7)	3 (1,6)	3 (2,5)
10–12 ($n = 240$)	6 (3,10)	4 (2,7)	3 (2,4)
13–22 ($n = 253$)	6 (3,11)	3 (1,8)	3 (2,4)
Missing ($n = 34$)	4 (2,6)	3 (2,6)	3 (2,4)
All ($n = 725$)	5 (3,9)	3 (2,7)	3 (2,4)

[a]Lower (Q1) and higher (Q3) quartiles.

not affected by age and sex, or by maternal age or education, or by infant feeding patterns.

Most case–control studies have found an inverse association between the age at introduction of cow's milk products in infancy and/or duration of breast-feeding and the risk of Type 1 DM.[5,27] However, in a recent US cross-sectional study of first degree relatives of subjects with Type 1 DM, 18 children with increased levels of IAA, GADA, and/or IA-2A did not differ from the autoantibody negative children ($n = 153$) in the age at introduction of cow's milk products or of cereals, fruits and vegetables, or meat.[28] The lack of evidence for an association between cow's milk exposure in infancy and Type 1 DM-associated autoantibodies in the study of Norris *et al.* and in the present larger series, as well as between early introduction of cow's milk and the development of clinical Type 1 DM in the present study, may be a consequence of a small number of cases. Furthermore, the risk ratios observed for Type 1 DM in case–control studies have been quite low, on average about 1.4.[5] Another factor that may contribute to the apparent discrepancy between the current findings and our previous observations, may be related to the fact that the children progressing to Type 1 DM in the present study population represent familial Type 1 DM, while about 90 % of the cases in our and others' case–control surveys have represented sporadic, i.e. non-familial, Type 1 DM. In an extensive comparison of familial and non-familial Type 1 DM, we observed that

Table 3. Prevalence and cumulative incidence of Type 1 DM associated autoantibodies and the incidence rate of Type 1 DM by sociodemographic factors in the siblings of children with diabetes

		Type 1 DM associated autoantibodies [a]		Type 1 DM
	Number (%) of children	Prevalence[b] per 100 at baseline (cases)	Cumulative incidence[c] per 100 (cases)	Incidence rate[d] per 1000 yr (cases)
All	725 (100)	13.6 (95)	7.5 (45)	6.1 (33)
Boys	338 (47)	13.9 (45)	8.6 (24)	6.2 (16)
Girls	387 (53)	13.4 (50)	6.5 (21)	5.9 (1.7)
Child's age (yr)				
0–4	134 (18)	12.3 (15)	9.3 (10)	9.0 (9)
5–9	259 (36)	13.1 (33)	7.8 (17)	7.3 (14)
10–14	205 (28)	15.8 (32)	6.5 (11)	5.1 (8)
15–24	127 (18)	12.4 (15)	6.6 (7)	2.1 (2)
Maternal age (yr)				
17–22	148 (20)	12.5 (18)	8.7 (11)	2.6 (3)
23–29	373 (51)	13.1 (47)	5.8 (18)	6.2 (17)
30–44	203 (28)	15.1 (29)	9.8 (16)	8.5 (13)
Length of maternal education (yr)				
6–9	198 (27)	12.0 (23)	8.3 (14)	2.6 (4)
10–12	240 (33)	11.5 (27)	6.8 (14)	3.3 (6)
13–22	253 (35)	15.8 (38)	6.9 (14)	10.1 (19)
Missing	34 (5)	22.6 (7)	12.5 (3)	15.9 (4)

[a]Autoantibodies measured from 697 children.
[b]For Type 1 DM-associated autoantibodies at the time of diagnosis of Type 1 DM in the index child ($n = 697$).
[c]For Type 1 DM associated autoantibodies follow-up from the diagnosis of Type 1 DM in the index child over a period of 4 years, among those initially seronegative ($n = 602$).
[d]For development of Type 1 DM observation from the diagnosis of Type 1 DM in the index child until 31 October 1995 ($n = 725$).

Table 4. Adjusted relative odds of seroconversion to positivity of Type 1 DM associated autoantibodies[a] and adjusted hazard ratio of development of Type 1 DM during follow-up[b] associated with nutritional factors (95 % confidence intervals in parentheses)

	Seroconversion		Type 1 DM	
	Odds ratio 1[c] (95 % CI)	Odds ratio 2[d] (95 % CI)	Hazard ratio 1[c] (95 % CI)	Hazard ratio 2[d] (95 % CI)
Duration of total breast-feeding				
≥2 months vs <2 months	0.70 (0.3–1.8)	0.58 (0.2–1.9)	0.49 (0.2–1.3)	0.53 (0.2–1.6)
Age at introduction of supplementary milk feeding				
<2 months vs ≥2 months	0.91 (0.4–2.1)	0.61 (0.2–1.6)	1.88 (0.7–4.9)	1.44 (0.5–4.2)
Childhood milk and sour milk consumption				
≥3 glasses vs <3 glasses	2.21 (0.9–5.2)	3.97 (1.3–11.7)	3.11 (1.1–9.3)	2.75 (0.9–8.4)
Length of maternal education				
10–12 yr vs <10 yr	0.84 (0.4–2.0)	0.95 (0.4–2.6)	1.45 (0.4–5.8)	1.80 (0.5–6.9)
>12 yr vs <10 yr	0.87 (0.4–2.1)	1.09 (0.4–3.0)	3.95 (1.3–12.5)	3.71 (1.1–12.1)

[a]For disease-associated autoantibodies follow-up from the diagnosis of Type 1 DM in the index child over a period of 4 years.
[b]For development of Type 1 DM observation from the diagnosis of Type 1 DM in the index child until 31 October 1995.
[c]For dietary variables odds ratio 1 and hazard ratio 1 adjusted for age, sex, and maternal age and education. For maternal education odds ratio 1 and hazard ratio 1 adjusted for age, sex, and maternal age.
[d]Odds ratio 2 and hazard ratio 2 adjusted for age, sex, maternal age, and for all variables in the table.

the familial cases were characterized by a stronger HLA DQB1 defined genetic susceptibility.[29] This raises the possibility that the role and impact of various exogenous factors triggering and/or potentiating beta-cell autoimmunity may be different between familial and sporadic Type 1 DM. However, we could not find any significant differences between familial and non-familial cases in the frequency and levels of the diabetes-associated autoantibodies at diagnosis which suggests a similar pathogenetic process in these two forms of Type 1 DM.

The results from case–control studies comparing cow's milk consumption during childhood are inconsistent: in a Swedish study the cases had consumed milk less frequently than the controls,[30] in our Finnish study no difference was observed in the amount of milk consumed by case and control children aged 0–6 years,[8] and in an Australian study from New South Wales cases had a higher intake of cow's milk protein than control children.[31]

Both cellular and humoral immune responses to cow's milk proteins have been found to be elevated in children with newly diagnosed Type 1 DM compared with non-diabetic control children or siblings.[9,32–34] Increased T-cell reactivity to β lactoglobulin and β casein but not to α casein has been observed in subjects with recent onset Type 1 DM,[34,35] the results on cellular immunity to bovine serum albumin being contradictory.[34,36,37] It has been shown that the cellular immune response to β lactoglobulin can be depressed in infants by delaying the exposure to cow's milk until 9 months of age.[38] Infant feeding patterns and milk consumption during childhood are related to cow's milk antibody titres. Duration of breast-feeding and age at introduction of dairy products were inversely associated with the titres of several cow's milk antibodies both in children with and without diabetes,[8,9] whereas milk consumption during childhood was directly related to cow's milk antibody titres.[8] It has been suggested that there is a dysregulation of oral tolerance in Type 1 DM which could have a pathogenic impact.[7]

Cow's milk has been shown to be diabetogenic in BB rats and NOD mice,[10,11] although not consistently.[39] A recent observation that dietary exposure also during adolescence causes diabetes in BB rats[39] contradicts an earlier observation which suggested that only the weaning period would be critical for the development of diabetes in BB rats.[40]

The incidence of Type 1 DM has more than tripled in Finland among children under 15 years of age over the last four decades.[1,2] During the same period the *per capita* consumption of liquid milk has substantially decreased from 333 litres per year in the 1950s to 217 litres in 1990.[41] However, the processing of milk has also changed, with pasteurization since 1946 and increasing in homogenization since the 1960s. Such processing modifies the characteristics of the milk,[42] e.g. homogenization reduces the size of fat globules to smaller particles which may permeate the intestine wall more readily.

In the present study, the consumption of sour milk products and cheese was not related to the emergence of disease-associated autoantibodies or to clinical Type 1 DM. This could be explained by the relatively small amounts consumed or by the differences in the protein structure.[42] The composition of milk has also changed with changes in breeds and feeding of cows and increasing use of low-fat and skim-milk.[43]

The registered amount of milk and sour milk consumed as a beverage in the present study seems to be valid. In a dietary survey performed in 17 municipalities in various parts of Finland in 1980, the average daily consumption of milk and milk products (including milk used in food preparation) varied between 510 g and 699 g for girls and between 627 g and 981 g for boys in the age range 3–18 years[14] and the amount of milk consumed varied only little between the age groups, in agreement with the present study.

The present prospective finding of a positive association between length of maternal education and risk of developing Type 1 DM contradicts some earlier findings from case–control studies.[44,45] Maternal education and cow's milk consumption during childhood were both associated with the risk of Type 1 DM independently of each other.

The present study is the first prospective study in which some evidence of an association has been observed between cow's milk consumption during childhood and seroconversion to positivity for Type 1 DM associated autoantibodies and progression to clinical Type 1 DM among siblings of children with Type 1 DM. This finding needs to be corroborated in other studies and also among unselected populations. Daily consumption of low-fat or skim-milk is encouraged in all dietary recommendations. Because of the importance of milk in a balanced diet in children, the study of putative diabetogenic mechanisms of cow's milk is urgent.

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